Acute and sub-acute toxicity of ethanolic extract of *Canthium mannii* Hiern stem bark on *Mus musculus*

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Acute and sub-acute toxicity of ethanolic extract (ETE) of *C. mannii* was assessed on white mice (*Mus musculus*). After 48 h of extract administration, no death was registered. It was deduced that the LD_{50} was indisputably higher than 16 g/kg body weight. The sub-acute toxicity test was based on the daily administration of three doses of ETE (300, 600 and 1200 mg/kg body weight) for four weeks; 1% DMSO served as negative control. As for the first experiment, no sign of toxicity was registered. Conversely, the sub acute doses stimulated and increased the weight-rate of mice after 7 days of treatment. Except for the spleen weight, the doses administrated did not modify the weight index. It was observed that, sub acute doses induced and increased (a) the food (particularly) and water consumption according to time and (b) the number of red and white blood cells. It was thought that, ETE can stimulate the haematopoietic function. Finally, no time variation of the activity of alanine aminotransferase and aspartate aminotransferase enzyme was observed in the serum of euthanized mice. The results showed the innocuity of ETE of *C. mannii* and thus validated his utilization in cameroonian traditional pharmacopoea.

**Keywords:** *Canthium mannii*, Stem-bark extract, Toxicity

During eighties, the World Health Organization noticed that, in both developed and poor countries, the populations have developed interest in phytotherapy because of the constant failure of modern medicine\(^1\). The increasing cost, non-availability of modern drugs, and limited access to adequate health care have compelled about 80% of the world population to use traditional pharmacopoea for primary health care especially in the tropical and sub-tropical regions\(^2\). About 75% of herbal drugs, such as morphine, ergotamine, vinblastine and digitaline used to treat different human affections have natural origin\(^3\). Most of these plants used and which are available in local markets have never been formally tested either for their efficacy or for their toxicity\(^4\). Besides this, the fact that a drug has a natural origin did not assure its innocuity. The use of a medicinal plant on its entire form, macerate, infusion, decoction, or on the form of cataplasm, unguent or expression can induce some side effects or allergic reactions of short, middle and long term type. One such plant namely *Canthium mannii* Hiern has the reputation as worm medicine and used traditionally by the people of Dschang, Westen Region of Cameroon (Central Africa). This plant is commonly called “A koup memia” in Yemba\(^4\), “kot missou” in Badjoue\(^5\) or “worm stem bark” in English. Traditionally the treatment consists of eating a portion of the stem bark of the shrub, sometimes with fried groundnuts, twice a day, for three days. Moreover, extracts of the bark of *C. mannii* showed nematicidal activities both *in vitro* and *in vivo* but their toxicity have never been investigated\(^6\). Keeping the above information in view, the present study has been undertaken to evaluate the acute and sub-acute toxicity of the ethanolic extract (ETE) of *C. mannii* on white mouse.

**Materials and Methods**

*Plant materials—* *Canthium mannii* (Rubiaceae) is a climbing shrub up to 6 m high and branch like glabrous\(^7\). Fresh *C. mannii* stem bark, collected in Fondonera, a village situated 25 km from Dschang

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(Western Region of Cameroon, Central Africa) was identified by the National Herbarium of Cameroon (NHC) and cut to small sizes (2 cm × 5 cm). They were air dried for 3 to 4 h daily for 7 days, grounded and stored in air tight plastic bags for about one week at room temperature (24°C) and 67% RH for subsequent use in the laboratory. Stored powder (400 g) was macerated in 2.5 liters of 95% ethanol. The mixture was stirred daily and after 72 h, this solution was filtered through a filter paper (pore size 2.5 µm) and the ethanolic extract was obtained. This was followed by the dilution of the concentrated extract. Dimethylsulfoxide (DMSO, Fluka-Analytical) was obtained from the chemical store of the Laboratory of Applied Biology and Ecology and manufactured by Sigma-Aldrich Chemie Gmbh.

Animals—White mice (Mus musculus) weighing 20-25 g body weight and aged between 7-8 weeks were obtained from the animal house of the Department of Animal Biology of the University of Dschang and were feed with animal feed.

Acute toxicity test—The mice were randomly divided into 5 groups of 6 animals each. They were allowed to acclimatize for one week before the beginning of the experiment. Mice were starved 24 h before oral administration of ETE and DMSO. They were divided into following 5 groups:

Gr. I: 6% DMSO (negative control), Gr. II: 2 g/kg body weight ETE, Gr. III: 4 g/kg body weight ETE, Gr. IV: 8 g/kg body weight ETE and Gr. V: 16 g/kg body weight ETE.

After 48 h of the oral administration of ETE and 6% DMSO, vivacity, sensibility to noise, sensibility to a painful stimulation, visual acuity and the aspect of the faeces were measured each hour. The LD$_{50}$ of ethanolic extract of C. mannii was investigated based on the eventual observed mortality.

Sub-acute toxicity test—In the second experiment, 4 groups of 6 animals each of the same age and weight were divided and housed under standard husbandry condition for one week before the experiment and allowed with standard feed in solid form (balls). Animals were divided into following 4 groups:

Gr. a: 1% DMSO (negative control), Gr. b: 300 mg/kg body weight ETE, Gr. c: 600 mg/kg body weight ETE and Gr. d: 1200 mg/kg body weight ETE.

The volume of ETE was measured using a manual syringe of 1 ml capacity with a blunt ended needle and the dose per each animal was slowly administered orally. All mice were weighed prior to treatment and given the drugs based on the individual body weight between 0700 and 0800 hrs. After this manipulation, mice were returned in their respective cages where they had access to food and water. They were treated for four weeks. At the beginning and during the treatment, in addition to physiological and behavioural parameters assessed in acute toxicity, the means weight of food and volume of water consumed were registered daily.

Red and white blood cells count—Red and white blood cells of each mouse were assessed three times (at day 0: before treatment, 14 days: during treatment and 29 days: post-treatment).

Since no death in experimental animals was registered during the treatment period, after 28 days, mice were euthanized using chloroform. The blood of each mouse was collected after decapitation in a tube without heparin. The serum was collected using a Pasteur pipette and was preserved in a refrigerator at 8°C for the estimation of serum glutamate pyruvate transaminase (SGPT/ALT) and the serum glutamate oxaloacetate transaminase (SGOT/AST) The enzymatic activity was assessed through the variation of the absorbance of the reactional medium (serum + reactive). Following the collection of the blood, the heart, the liver, the lungs, the spleen, the kidneys and the gastro-intestinal tract (GIT) of each mouse were also collected, weighed and their external features were compared to those of the control group under a dissecting microscope.

Statistical analysis—Results were expressed as mean± SE. Significance was evaluated using 1 or 2-ways analysis of variance (ANOVA) and paired T test and considered to be statistically significant at $P < 0.05$.

Results

Acute toxicity—Following the administration of single dose of 2, 4, 8 and 16 g/kg body weight of ETE to mice, no death was registered after 48 h post-treatment.

Sub-acute toxicity—At the end of the fourth week of experiment at day 28 no variation of the vivacity, sensibility to noise and to painful stimulation, visual acuity and consistency of faeces was noted in both experimental and control groups. Moreover, no additional death or modification of the pigmentation of the viscera was observed. In different treated groups, the mean weight rate was high at the end of the first week, i.e. at day 7 (Fig. 1). This rate was at
least greater than and equal to 2% between day 14 and day 28 and significant different ($P < 0.05$) of that obtained at day 7. Overall, no significant ($P > 0.05$) variation of the mean weight rate was observed from day 14 between the tested doses of ETE. Except the weight index of the spleen, the variation of the dose did not significantly modify the weight index of the organs. With the high dose we obtained 0.86 ± 0.45, 7.48 ± 3.55, 1.92 ± 2.98, 2.14 ± 1.92 for the weight index of heart, liver, lungs and kidney respectively. For the spleen, it was noticed that, the spleen index reduced with the increase of the ETE dose. This index was proportionally inverted to that of the lungs. However, this variation was not statistically significant ($P > 0.05$). In general, water intake and particularly food intake increased with time and with the dose of ETE absorbed (Table 1) but this observation was not significant ($P > 0.05$). Despite an increase in tendency of the density of red and white blood cells between day 0 and day 28 according to the doses of ETE absorbed, these variations were not statistically significant ($P > 0.05$; Fig. 2 a and b). This results showed a slight variation of the absorbance between groups receiving different doses of ETE. For AST the absorbance obtained were 6.14 ± 0.65, 9.06 ± 11.32 ± 2.59 and 12.62 ± 1.62 U/L and for ALT and 7.44 ± 2.59, 1.33 ± 2.59, 14.23 ± 1.62, 14.88 ± 1.62 U/L for 1% DMSO (negative control), 300 mg.kg$^{-1}$, 600 mg.kg$^{-1}$ and 1200 mg.kg$^{-1}$ respectively. Differences between treated groups compared to negative control were significant ($P < 0.05$). However, the absorbance did not vary according to time in the serums of euthanized mice.

**Discussion**

The maximum acute dose administrated (16 g of ETE/kg body weight) was six time greater than 2.5 g/kg body weight recommended during the screening of plant toxicity$^{13}$. For a comparison, it was found that the LD$_{50}$ of mebendazole was 640 mg/kg body weight in mouse$^{14}$. The results of the present study suggest the innocuity of the consumption of the stem bark extract of *C. mannii*. The maximum dose administrated comprised between 10 and 20 g/kg body weight is high and not compatible with therapeutic doses$^{15}$. By this innocuity, ETE of *C. mannii* can be ranged at the side of water extract of

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**Table 1**—Variation of mean quantity of food (feed and water) consumed by mice following oral administration of ethanolic extract of *C. mannii* [Values are mean ± SE from 6 mice in each group]

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Feed (g)</th>
<th>Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO (1%)</td>
<td>D$_{1-7}$</td>
<td>D$_{8-14}$</td>
</tr>
<tr>
<td>300</td>
<td>08.78±4.06$^a$</td>
<td>10.71±2.14$^a$</td>
</tr>
<tr>
<td>600</td>
<td>07.14±3.08$^a$</td>
<td>12.21±1.87$^a$</td>
</tr>
<tr>
<td>1200</td>
<td>10.28±4.70$^a$</td>
<td>14.28±3.20$^a$</td>
</tr>
</tbody>
</table>

In each column, number with the same superscript did not significantly different ($P > 0.05$; two ways ANOVA), ETE= Ethanotic extract, DMSO= dimethylsulfoxide.
Achyrocline satureoides\textsuperscript{16} or of Ajuga iva used in Morocco as a panacea in traditional medicine\textsuperscript{17}. The oral administration of extracts of these plants to mice at single doses of 2 to 14 g/kg and daily dose of 10 mg/kg respectively to rats during two weeks did not induce any side effects\textsuperscript{17}. Conversely to ETE of \textit{C. mannii}, some plants extracts showed toxic effects. For example, water extract of \textit{Albizia anthelmintica} administrated to mice at the dose of 0.5 g/kg body weight caused 100\% mortality. In the same way, the essential oil of \textit{Allium sativum} administrated at dose of 100 mg/kg body weight was toxic to male rats\textsuperscript{18}.

Eventhough acute toxicity helps to classify the substances that are non-toxic, it is important to assess the sub-acute toxicity because it helps to evaluate the morphological and physiological changes of organs. The mean gain in weight was high in groups which received ETE may be due to the beneficial effect of the extract. After the consumption of ETE, animals showed a slight stimulation of appetite. It is known that in addition to their therapeutic properties, medicinal plants can affect positively the nutrition of an animal\textsuperscript{19}. The density of blood cells in animals receiving the ETE showed a relative increase compared to those of control group. It was thought that ETE can stimulate the haematopoietic function of the animals. In case of white blood cells, this plant could help people with immune depressive system in some diseases. The fact that, the enzymatic activity did not vary according to time in animals receiving sub-acute doses of ETE confirmed the innocuity of this extract. In conclusion, the administration of acute doses of ETE did not cause mortality. Also, the sub-acute doses did not affect negatively the physiological and behavioural parameters of mice. The ETE relatively increased the number of white and red blood cells, favoured weight rate increase and the appetite of animals. Therefore, it is thought that ETE may play a role in the reconstitution or in the building of cells.

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